

AD _____

Award Number: DAMD17-00-1-0265

TITLE: Growth Factor Regulation of an Angiogenic Factor, the Fibroblast Growth Factor-Binding Protein (FGF-BP), in Breast Cancer

PRINCIPAL INVESTIGATOR: Benjamin L. Kagan

CONTRACTING ORGANIZATION: Georgetown University Medical Center
Washington, DC 20057

REPORT DATE: August 2001

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20020118 197

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE August 2001	3. REPORT TYPE AND DATES COVERED Annual Summary (01 Aug 00 - 31 Jul 01)	
4. TITLE AND SUBTITLE Growth Factor Regulation of an Angiogenic Factor, the Fibroblast Growth Factor-Binding Protein (FGF-BP), in Breast Cancer			5. FUNDING NUMBERS DAMD17-00-1-0265	
6. AUTHOR(S) Benjamin L. Kagan				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Georgetown University Medical Center Washington, DC 20057 E-Mail: kaganb@georgetown.edu			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited				12b. DISTRIBUTION CODE
13. Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information) A secreted carrier protein has been described which is able to bind to FGF-1 and FGF-2 in a non-covalent, reversible manner. FGF-2 bound to this protein was not subject to degradation and retained its mitogenic activity. This FGF-binding protein (FGF-BP) has been studied extensively by our laboratory. FGF-BP is highly expressed in squamous cell carcinomas (SCC) and EGF is able to increase the expression of FGF-BP in SCC derived cell lines through PKC, MEK/ERK, and p38 MAPK signaling. We have found FGF-BP mRNA to be expressed in two breast cancer cell lines (MDA-MB-468, MCF-7/ADR), by Northern Analysis/Ribonuclease Protection. EGF treatment of MDA-MB-468 cells resulted in an increase in FGF-BP mRNA expression in a time-dependent manner. EGF signaling occurs primarily through the PKC, and p38 MAPK pathways. Finally, EGF induction of the FGF-BP promoter is mediated through CCAAT/enhancer binding protein and AP-1 transcription factor binding sites on the promoter.				
14. SUBJECT TERMS Growth Factors, Ribonuclease Protection, Transcription, DNA-Protein Interactions, Angiogenesis				15. NUMBER OF PAGES 11
				16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

Table of Contents

Cover.....	1
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	6
Reportable Outcomes.....	7
Conclusions.....	7
References.....	7
Appendices.....	10

Annual report for Grant Number DAMD17-00-1-0265
August 1, 2000 to July 31, 2001

P.I.: Benjamin L. Kagan

Title: Growth Factor Regulation of an Angiogenic Factor, the Fibroblast Growth Factor-Binding Protein (FGF-BP), in Breast Cancer

I. Introduction

Paracrine and autocrine growth factors have many functions, including a crucial role in inducing the formation of new blood vessels in a healing wound, as well as in a growing tumor. Many studies have demonstrated that a solid tumor mass cannot grow beyond a few millimeters in size without a sufficient supply of blood to the tumor. Tumor blood vessels provide a pathway for tumor cells to metastasize to distal sites, as well as a source of nourishment [1-4]. The most important and best-studied angiogenesis factors belong to the family of fibroblast growth factors (FGFs) [5, 6]. FGF-1 and FGF-2 (aFGF and bFGF, respectively) are unique in that their biological activities can be quenched by binding tightly to heparansulfate proteoglycan molecules in the extracellular matrix [7-10]. Two alternate mechanisms of FGF-1 and FGF-2 activation have been theorized as a result of a multitude of studies over the last decade. One mechanism involves the solubilization of FGF-2 from its storage site by heparanase digestion of the glycosaminoglycan portion of the cell attachment [11-14]. The second mechanism involves the binding of FGF to a secreted carrier protein delivering the activated FGF to its target receptor. A secreted carrier protein has been described which is able to bind to FGF-1 and FGF-2 in a non-covalent, reversible manner [15]. FGF-2 bound to this protein was not subject to degradation and retained its mitogenic activity [15]. This **FGF-binding protein (FGF-BP)** has been studied extensively by our laboratory.

Expression of FGF-BP in cell lines that express FGF-2 results in these cells having a tumorigenic and angiogenic phenotype [16]. FGF-BP transfected cells have been shown to release the protein into their media along with FGF-2 in a non-covalently bound form; the released FGF-2 is now biologically active [17]. FGF-BP mRNA is expressed in SCC, colon, and breast tumor cell lines and primary tumor tissue [16]. The role of FGF-BP during tumor progression has been studied by our laboratory using skin carcinogenesis as a model for epithelial cancers. We have shown that FGF-BP mRNA is upregulated in the skin during mouse development, but drops to low levels in adult mouse skin. In both mouse and human skin, FGF-BP mRNA and protein levels increase at least 3-fold upon treatment with PKC-activating TPA (12-*O*-tetradecanoylphorbol-13-acetate), and increase further in DMBA/TPA induced papillomas and carcinomas [18].

II. Body

The human FGF-BP promoter was recently isolated and cloned revealing positive and negative regulatory elements within a 118 base pair region just upstream of the FGF-BP transcription start site (**Figure 1**). The phorbol ester TPA was then shown to upregulate FGF-BP transcription in ME180 squamous cell carcinoma cells. This transcription was mediated through the activation of protein kinase C, and the Sp1, AP-1, and C/EBP positive regulatory elements in the FGF-BP promoter [19]. Treatment of ME180 SCC cells resulted in the upregulation of FGF-BP mRNA. Signal transduction was mediated through the EGFR, PKC, MEK/ERK, and p38 pathways, while transcription was mediated through the AP-1 and C/EBP regulatory elements in the promoter [20]. Finally, we have also shown that serum upregulates FGF-BP expression in ME180 cells, predominantly through PKC and p38 signaling, while only through the C/EBP site on the FGF-BP promoter [21]. **In my accepted proposal, I hypothesized that an angiogenic "funneling" effect exists in which intracellular signals initiated by EGF and related ligands result in the activation of FGF through the**

modulation of the FGF-BP gene. I planned to consider the relevance of this “funneling” effect with respect to the breast cancer system.

We have found FGF-BP mRNA to be expressed in two breast cancer cell lines, and 4 out of 6 clinical samples of human breast cancers, by Northern Analysis/Ribonuclease Protection, and RT-PCR, respectively. We have also detected FGF-BP mRNA in the human and mouse mammary gland. **This report summarizes the findings by Benjamin Kagan as PI of the funded research, testing the role of FGF-BP in human breast cancer cell progression and its regulation by the epidermal growth factor.**

Aim 1: To study the regulation of the FGF-BP mRNA, by growth factors, in breast cancer.

Detection of endogenous FGF-BP mRNA in MCF-7/ADR and MDA-MB-468 human breast cancer cell lines. Previously, we were able to show that FGF-BP mRNA was expressed in 9 out of 15 breast cancer cell lines, by RT-PCR. To study the regulation of FGF-BP expression in breast cancer cell lines, we wanted to use a quantitative method for detection of FGF-BP mRNA. A ribonuclease protection assay specific for human FGF-BP was developed using a riboprobe derived from a pRC/CMV vector plasmid containing the FGF-BP open reading frame [16]. We were able to detect FGF-BP mRNA only in the MCF-7/ADR cell line, an adriamycin resistant clone of the MCF-7 cell line, as well as the ME180 SCC cell line, which was used as a positive control. Northern analysis was also used, screening a wider array of breast cancer cell lines. **We were able to detect expression of FGF-BP mRNA in both MCF-7/ADR and the MDA-MB-468 cell lines. Expression of FGF-BP mRNA, as determined by RNase protection and Northern analysis, is summarized in Table 1.**

EGF regulation of endogenous FGF-BP in MDA-MB-468 cells. Studies have shown that the MDA-MB-468 cell line overexpresses the EGFR as compared to MCF-7 breast cancer cells [22-24]. Biscardi et al. [24] measure levels of EGFR to be 35 fold that of MCF-7 cells. Because the MDA-MB-468 cell line, like the ME180 cell line, express high levels of the EGFR [22], we decided to test whether FGF-BP mRNA expressed in these cells can be regulated by EGF and/or TPA. MDA-MB-468 cells were grown to 80% confluency, serum starved for 24 hours, and treated with EGF for 1, 3, 6, or 24 hours. FGF-BP mRNA levels were analyzed by Northern analysis, and we were able to observe that EGF induced FGF-BP upregulation at about 3-fold above control, peaking at 6 hours of EGF treatment (**Figure 2**). The time-course of EGF induction of FGF-BP mRNA in MDA-MB-468 cells was similar to that observed in the ME180 SCC cell line, suggesting similar mechanisms of regulation [20]. **These data demonstrate that EGF can regulate FGF-BP in MDA-MB-468 cells, in a similar manner to ME180 SCC cells**

EGF induction of FGF-BP in MDA-MB-468 cells is mediated through PKC and p38 MAPK signaling. EGF regulation of FGF-BP mRNA in ME-180 cells occurs through PKC, and the MEK/ERK and p38 MAPK signaling pathways [20]. Serum, in contrast, mediates FGF-BP transcription through PKC and p38 MAPK signaling, but not MEK/ERK [21]. To discern between the possible signaling pathways involved in EGF induction of FGF-BP in MDA-MB-468 cells, we tested pharmacological inhibitors of signal transduction at various concentrations for their affect on FGF-BP regulation. We found that treatment with the EGFR tyrosine kinase inhibitor PD153035 resulted in a significant concentration dependent inhibition of EGF induction of FGF-BP mRNA (**Figure 3**). Therefore, as expected, EGFR tyrosine kinase activity is essential for the EGF effect. To establish whether PKC activation was also required for the EGF effect on FGF-BP, we treated MDA-MB-468 cells with the bisindolylmaleimide PKC inhibitor Ro 31-8220 [25]. At concentrations of 1 μ M and 10 μ M, Ro 31-8220 was able to significantly inhibit the EGF induction of FGF-BP (**Figure 3**). At these concentrations Ro 31-8220 is also able to inhibit other kinases including the mitogen- and stress-activated protein kinase-1 (MSK1) [26], therefore we tested whether the PKC-specific inhibitor calphostin C [27] could also inhibit the EGF effect. Treatment with 100 nM calphostin C significantly reduced EGF-induced FGF-BP

mRNA expression by 50% (**Figure 3**). Taken together, these data suggest a role for PKC in the EGF induction of FGF-BP in MDA-MB-468 cells.

To determine whether different MAP kinase pathways were also involved in the EGF effect on FGF-BP, we used the MEK1/2 specific inhibitor U0126 and the p38 MAPK specific inhibitor SB202190 [28, 29]. Treatment with 1 μ M and 10 μ M U0126 did not significantly inhibit EGF induction (**Figure 3**). Although 20 μ M U0126 significantly inhibited EGF induction of FGF-BP, the overall inhibition was only around 30% as compared to the ability of U0126 to inhibit the EGF induction of FGF-BP in ME-180 cells by 70% [20]. This suggests a lesser role for the MEK/ERK pathway in the EGF effect in MDA-MB-468 cells. In contrast, as seen in the ME-180 cells, treatment with increasing concentrations of the p38 MAPK inhibitor SB202190, resulted in a concentration-dependent inhibition of EGF-induced FGF-BP mRNA expression ranging from 55% inhibition at 5 μ M to 80% inhibition at 20 μ M. Furthermore, as described above, the bisindolylmaleimide Ro 31-8220 was able to significantly inhibit EGF-induced FGF-BP mRNA expression at concentrations specific for PKC and other kinases such as MSK1. MSK1 has been shown to be activated by p38 MAPK phosphorylation [26, 30]. Taken together, these data suggest that p38 MAPK plays a dominant role in the induction of FGF-BP by EGF in MDA-MB-468 cells.

Other intracellular targets for EGF receptor-induced intracellular signaling include members of the c-Src protein tyrosine kinase family. c-Src family members interact with the EGFR at tyrosine residues via SH2 domains [31]. MDA-MB-468 cells have been shown to express moderate levels of c-Src protein as compared normal breast epithelium [24]. Therefore, we used the c-Src family specific inhibitor PP1 [32]. Treatment with PP1 resulted in a maximal inhibition of EGF induction of FGF-BP of 20% only at the highest concentration, 10 μ M (**Figure 3**). Concentrations of 1 μ M and 0.1 μ M, also shown to inhibit s-Src family members [32], had no effect. This suggests that c-Src family members do not play a role in the EGF effect.

Aim 2: To study the regulation of the human FGF-BP promoter in breast cancer cells.

EGF regulation of the FGF-BP promoter in MDA-MB-468 cells. As described above, EGF induces the upregulation of FGF-BP in MDA-MB-468 breast cancer cells. To determine if this regulation occurred at the transcriptional level, we tested whether EGF regulated the activity of FGF-BP promoter in MDA-MB-468 cells. As described above, various portions of the human FGF-BP promoter, full-length, mutated, or deleted, have been cloned upstream of a luciferase reporter gene. These constructs have been used successfully to assess the activity of the FGF-BP promoter in ME180 cells [19, 20, 33]. We were able to show that in MDA-MB-468 cells, treatment with EGF was able to induce the activity of the -1060/+62 and -118/+62 promoter constructs 4- to 5-fold above basal (**Figure 4**). Deletion of either the AP-1 or the C/EBP, and not the Sp1(b) site, reduced the induction by EGF of the promoter constructs, suggesting the AP-1 and the C/EBP sites were necessary for EGF induced FGF-BP transcription in this cell line. This observation is similar to what was observed in the ME180 cells [20]. Upon further investigation, cell-type specific differences were observed. Deletion of the AP-1 site resulted in a statistically significant decrease in promoter basal activity, suggesting the AP-1 site is necessary for basal activity. Deletion of the C/EBP site revealed a statistically significant increase in promoter basal activity, suggesting differences in C/EBP binding to the site affecting both basal and EGF induced activity of the FGF-BP promoter. **These data show that EGF is able to induce the activity of the FGF-BP promoter in MDA-MB-468 cells, through the AP-1 and C/EBP sites, as seen in ME180 cells. In addition, C/EBP binding to the FGF-BP promoter may repress basal activity while enhancing promoter activity after EGF treatment.**

III. Key Research Accomplishments

- Expression of FGF-BP mRNA was detected in both MCF-7/ADR and the MDA-MB-468 cell lines by Northern analysis and RNase protection.
- EGF upregulates FGF-BP expression in MDA-MB-468 cells, in a similar manner to ME180 SCC cells. This occurs predominantly through the PKC and p38 MAPK signaling pathways.

- EGF is able to induce the activity of the FGF-BP promoter in MDA-MB-468 cells, through the AP-1 and C/EBP sites, as seen in ME180 cells. Deletion of the C/EBP site on the FGF-BP promoter results in a significant increase in basal promoter activity.

IV. Reportable Outcomes

Manuscripts, abstracts, and publications produced as a result of this funded research:

Kagan BL, Harris VK, Coticchia CM, Ray R, Wellstein A and Riegel AT, Transcriptional regulation of a binding protein for FGF (FGF-BP) through p38/SAPK2 signaling. In: *Proceedings of the American Association for Cancer Research, New Orleans, LA, March 24-March 28, 2001.*

Kagan BL, Cabal-Manzano R, Stoica GE, Nguyen Q, Wellstein A, and Riegel AT, EGF-induced fibroblast growth factor-binding protein (FGF-BP) expression in breast cancer is mediated through C/EBP β -regulated transcription and p38 MAPK signaling. 2001 (manuscript in preparation)

V. Conclusions

We were able to observe FGF-BP expression in the MDA-MB-468 cell line. In this model we demonstrated that EGF was able to upregulate FGF-BP transcription. This is important in the context of breast cancer because expression of the EGFR has been inversely correlated with ER expression, and along with expression of the EGFR family member HER2, the EGFR has been correlated with a poor prognosis for breast cancer. FGF-BP expression, and its regulation by EGF in the MDA-MB-468 breast cancer cell line, may suggest that FGF-BP plays a role in the expression of a more angiogenic phenotype in breast cancer.

As described above, deletion of the C/EBP site on the FGF-BP promoter resulted in a significant increase in promoter basal activity. This suggests that a C/EBP factor binding to this site acts as a repressor. Recently, a variant of C/EBP β , the liver enriched inhibitory protein (LIP), translated from the same mRNA as the full length protein (also called liver enriched activating protein or LAP), has been described [34, 35]. LIP is similar to LAP, except that it does not contain a transactivating domain. The LIP-LAP dimer is able to bind to its normal consensus site on a promoter, with greater affinity than LAP-LAP dimers, but is not able to promote transcription, therefore acting as a dominant negative [35]. LIP has also been found to be expressed in human breast cancer samples that are both ER and PR negative [36]. **These data suggest the possibility that LIP may be present on the FGF-BP promoter in the MDA-MB-468 cells under basal conditions acting as a repressor of FGF-BP basal activity. When stimulated with EGF, the C/EBP dimer might change to a LAP/LAP dimer and therefore enhance FGF-BP promoter activity.** These hypotheses are currently under investigation.

References

1. Folkman, J. and M. Klagsburn, *Angiogenic factors*. Science, 1987. 235(4787): p. 442-7.
2. Liotta, L.A., P.S. Steeg, and W.G. Stetler-Stevenson, *Cancer metastasis and angiogenesis: an imbalance of positive and negative regulation*. Cell, 1991. 64(2): p. 327-36.
3. Folkman, J., *How is blood vessel growth regulated in normal and neoplastic tissue? G.H.A. Clowes memorial Award lecture*. Cancer Res, 1986. 46(2): p. 467-73.
4. Fidler, I.J. and L.M. Ellis, *The implications of angiogenesis for the biology and therapy of cancer metastasis*. Cell, 1994. 79(2): p. 185-8.
5. Baird, A. and M. Klagsbrun, *The fibroblast growth factor family*. Cancer Cells, 1991. 3(6): p. 239-43.

6. Gospodarowicz, D., et al., *Structural characterization and biological functions of fibroblast growth factor*. Endocr Rev, 1987. 8(2): p. 95-114.
7. Vlodavsky, I., et al., *Endothelial cell-derived basic fibroblast growth factor: synthesis and deposition into subendothelial extracellular matrix*. Proc Natl Acad Sci U S A, 1987. 84(8): p. 2292-6.
8. Rogelj, S., et al., *Basic fibroblast growth factor is an extracellular matrix component required for supporting the proliferation of vascular endothelial cells and the differentiation of PC12 cells*. J Cell Biol, 1989. 109(2): p. 823-31.
9. Saksela, O., et al., *Endothelial cell-derived heparan sulfate binds basic fibroblast growth factor and protects it from proteolytic degradation*. J Cell Biol, 1988. 107(2): p. 743-51.
10. Kiefer, M.C., et al., *Ligand-affinity cloning and structure of a cell surface heparan sulfate proteoglycan that binds basic fibroblast growth factor*. Proc Natl Acad Sci U S A, 1990. 87(18): p. 6985-9.
11. Vlodavsky, I., et al., *Heparan sulfate degradation in tumor cell invasion and angiogenesis*. Adv Exp Med Biol, 1988. 233: p. 201-10.
12. Bashkin, P., et al., *Basic fibroblast growth factor binds to subendothelial extracellular matrix and is released by heparitinase and heparin-like molecules*. Biochemistry, 1989. 28(4): p. 1737-43.
13. Moscatelli, D., *Basic fibroblast growth factor (bFGF) dissociates rapidly from heparan sulfates but slowly from receptors. Implications for mechanisms of bFGF release from pericellular matrix*. J Biol Chem, 1992. 267(36): p. 25803-9.
14. Vlodavsky, I., et al., *Sequestration and release of basic fibroblast growth factor*. Ann N Y Acad Sci, 1991. 638: p. 207-20.
15. Wu, D.Q., et al., *Characterization and molecular cloning of a putative binding protein for heparin-binding growth factors*. J Biol Chem, 1991. 266(25): p. 16778-85.
16. Czubyko, F., et al., *Tumor growth and angiogenesis induced by a secreted binding protein for fibroblast growth factors*. J Biol Chem, 1994. 269(45): p. 28243-8.
17. Czubyko, F., et al., *A secreted FGF-binding protein can serve as the angiogenic switch in human cancer*. Nat Med, 1997. 3(10): p. 1137-40.
18. Kurtz, A., et al., *Expression of a binding protein for FGF is associated with epithelial development and skin carcinogenesis*. Oncogene, 1997. 14(22): p. 2671-81.
19. Harris, V.K., et al., *Phorbol ester-induced transcription of a fibroblast growth factor-binding protein is modulated by a complex interplay of positive and negative regulatory promoter elements*. J Biol Chem, 1998. 273(30): p. 19130-9.
20. Harris, V.K., et al., *Induction of the Angiogenic Modulator Fibroblast Growth Factor-binding Protein by Epidermal Growth Factor Is Mediated through Both MEK/ERK and p38 Signal Transduction Pathways*. J Biol Chem, 2000. 275(15): p. 10802-10811.
21. Harris, V.K., et al., *Serum induction of the fibroblast growth factor-binding protein (FGF- BP) is mediated through ERK and p38 MAP kinase activation and C/EBP- regulated transcription*. Oncogene, 2001. 20(14): p. 1730-8.
22. Filmus, J., et al., *MDA-468, a human breast cancer cell line with a high number of epidermal growth factor (EGF) receptors, has an amplified EGF receptor gene and is growth inhibited by EGF*. Biochem Biophys Res Commun, 1985. 128(2): p. 898-905.
23. Buick, R.N., J. Filmus, and J. Church, *Studies of EGF-mediated growth control and signal transduction using the MDA-MB-468 human breast cancer cell line*. Prog Clin Biol Res, 1990: p. 179-91.
24. Biscardi, J.S., A.P. Belsches, and S.J. Parsons, *Characterization of human epidermal growth factor receptor and c-Src interactions in human breast tumor cells*. Mol Carcinog, 1998. 21(4): p. 261-72.
25. Davies, S.P., et al., *Specificity and mechanism of action of some commonly used protein kinase inhibitors*. Biochem J, 2000. 351(Pt 1): p. 95-105.
26. Deak, M., et al., *Mitogen- and stress-activated protein kinase-1 (MSK1) is directly activated by MAPK and SAPK2/p38, and may mediate activation of CREB*. Embo J, 1998. 17(15): p. 4426-41.

27. Kobayashi, E., et al., *Calphostins (UCN-1028), novel and specific inhibitors of protein kinase C. I. Fermentation, isolation, physico-chemical properties and biological activities.* J Antibiot (Tokyo), 1989. 42(10): p. 1470-4.
28. Cuenda, A., et al., *SB 203580 is a specific inhibitor of a MAP kinase homologue which is stimulated by cellular stresses and interleukin-1.* FEBS Lett, 1995. 364(2): p. 229-33.
29. Kumar, S., et al., *Novel homologues of CSBP/p38 MAP kinase: activation, substrate specificity and sensitivity to inhibition by pyridinyl imidazoles.* Biochem Biophys Res Commun, 1997. 235(3): p. 533-8.
30. Rolli, M., et al., *Stress-induced stimulation of early growth response gene-1 by p38/stress-activated protein kinase 2 is mediated by a cAMP-responsive promoter element in a MAPKAP kinase 2-independent manner.* J Biol Chem, 1999. 274(28): p. 19559-64.
31. Biscardi, J.S., et al., *c-Src-mediated phosphorylation of the epidermal growth factor receptor on Tyr845 and Tyr1101 is associated with modulation of receptor function.* J Biol Chem, 1999. 274(12): p. 8335-43.
32. Amoui, M., P. Draber, and L. Draberovala, *Src family-selective tyrosine kinase inhibitor, PP1, inhibits both Fc epsilonRI- and Thy-1-mediated activation of rat basophilic leukemia cells.* Eur J Immunol, 1997. 27(8): p. 1881-6.
33. Harris, V.K., et al., *Mitogen-induced expression of the fibroblast growth factor-binding protein is transcriptionally repressed through a non-canonical E-box element.* J Biol Chem, 2000. 275(37): p. 28539-48.
34. Descombes, P., et al., *LAP, a novel member of the C/EBP gene family, encodes a liver-enriched transcriptional activator protein.* Genes Dev, 1990. 4(9): p. 1541-51.
35. Descombes, P. and U. Schibler, *A liver-enriched transcriptional activator protein, LAP, and a transcriptional inhibitory protein, LIP, are translated from the same mRNA.* Cell, 1991. 67(3): p. 569-79.
36. Zahnow, C.A., et al., *Overexpression of C/EBPbeta-LIP, a naturally occurring, dominant-negative transcription factor, in human breast cancer.* J Natl Cancer Inst, 1997. 89(24): p. 1887-91.

Appendix:

Figure 1. Regulatory region of the FGF-BP promoter

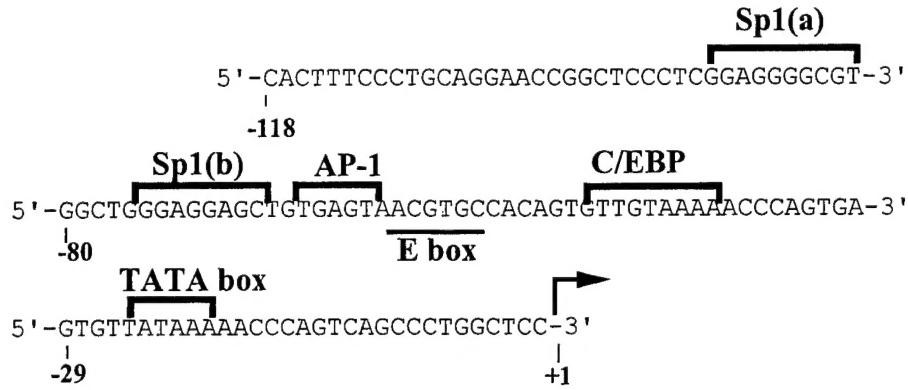


Table 1. Levels of FGF-BP, ER, and EGFR in human breast cancer cell lines. (adapted from Biscardi et al., *Mol Carcinog* 21:261-272, 1998)

Cell Line	EGFR	ER	FGF-BP
MCF-7	+	++	-
MCF-7/ADR	+++	-	++
BT20	++	-	-
BT474	-	+	-
T47D	-	+	-
T47Dco	-	-	-
MDA-MB 231	++	-	+/-
MDA-MB 468	++++	-	+++
BT549	++	-	+/-
Hs 578T	++	-	+/-

Figure 2. EGF induction of FGF-BP mRNA in MDA-MB-468 cells.

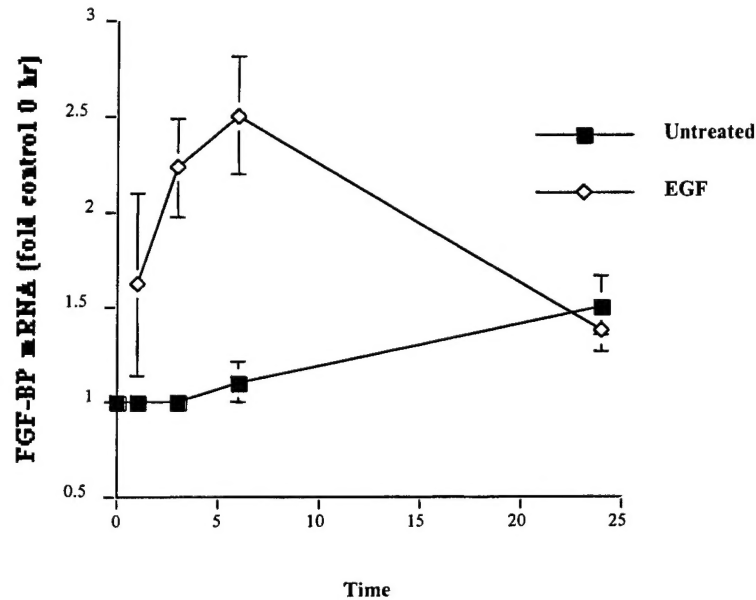


Figure 3. Effect of signal transduction inhibitors on the EGF induction of FGF-BP in MDA-MB-468 cells. The following inhibitors were used: Calphostin C (PKC), PD153035 (EGFR), U0126 (MEK1/2), PP1 (c-Src), Ro 31-8220 (PKC, MSK1), and SB202190 (p38/MAPK).

